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ARMSTRONG LABORATORY**

**TRICHLOROETHYLENE RADICALS:
AN EPR/SPIN TRAPPING STUDY**

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The animal use described in this study was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1966, as amended.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE DIRECTOR



STEPHEN R. CHANNEL, Maj, USAF, BSC
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13. ABSTRACT (Maximum 200 words) Trichloroethylene (TCE) is an environmental contaminant found in the soil and groundwater on several active bases as well as bases scheduled for closure. TCE is a halocarbon and is believed to cause environmental and biological damage through production of free radicals. As part of the process to develop environmental and health effects criteria for base clean-up, the initial radicals produced by TCE were studied by electron paramagnetic resonance spectroscopy (EPR). Radicals of TCE were formed by γ -radiation in a ^{60}Co γ -ray source. TCE at 77°K was irradiated at a dose rate of 1 Gy/min receiving a total dose of 10 By. The TCE radicals were detected with and without the spin traps 5,5-dimethyl-1-pyrroline-1-oxide (DMPO), N-tert-butyl- α -phenyl nitron (PBN) and 3,5-dibromo-4-nitrosobenzenesulphonate (DBNBS). The structure of the spin trapped TCE radical was $\text{Cl}_2\text{C}=\text{CH}\bullet$. The spin trap of choice for the study of radicals by EPR in biological tissue is PBN. The efficiency of PBN to trap the TCE radical was 20.6%.				
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PREFACE

This is one of a series of technical reports describing results of the experimental laboratory programs conducted for the USAF Environmental Initiative Program managed by Maj Stephen R. Channel. The research described in this report was performed at the Armed Forces Radiobiology Research Institute (AFRRI), Bethesda MD. This was collaborative research with Dr. A.J. Carmichael, Manager, EPR Facility, AFRRI. The work described was performed by Maj Steel-Goodwin while on permissive TDY to AFRRI, 1993. These results were presented at two divisional seminars sponsored by OL/AL HSC/OET at Wright-Patterson AFB, 1994. It provides the initial data required for proof of principal of electron paramagnetic resonance (EPR) technology prior to submission of any protocol to quantitate TCE-induced free radicals using this technique in biological samples.

The authors gratefully wish to thank Lt Col Terry A. Childress who serves as Contract Technical Monitor for the U.S. Air Force, Armstrong Laboratory, Toxicology Division and Dr. John Ainsworth, Scientific Director, Armed Forces Radiobiology Research Institute, Bethesda MD for permitting this collaborative research to be performed.

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ABBREVIATIONS

C	celsius
dB	decibel
DBNBS	3,5-dibromo-4-nitrosobenzenesulphonate
DMPO	5,5-dimethyl-1-pyrroline-1-oxide
EPR	electron paramagnetic resonance
eV	electron volts
g	gram
Gy	Gray
K	kelvin
L	liter
mg	milligram
mM	millimole/L
PBN	N-tert-butyl-α-phenyl nitron
TCE	trichloroethylene

SECTION 1

INTRODUCTION

Free radical reactions are a natural process in biological systems. For example, superoxide($O_2^{\bullet-}$) and nitric oxide (NO^{\bullet}) are produced beneficially as part of various biochemical pathways in many cells (Carmichael et al 1993). In the normal functioning cell these radicals are in harmony and their levels are balanced. However, when this balance is altered by induction of one of these species, or by the presence of other free radicals (e.g. carbon-centered radicals) generated as metabolites of xenobiotic agents, a chain of events may occur through the propagation of free radical pathways which ultimately lead to cell transformation and/or death.

The general objective of this research was to study toxic free radical mechanisms induced in biological systems following exposure to trichloroethylene, (Gonthier and Barret 1989) and other chemicals or their metabolites known to follow free radical pathways (such as carbon tetrachloride (Knecht & Mason 1988, LaCagnin et al 1988, and Sentjurc and Mason 1992) and ammonium dinitramide (Pace 1994). Free radicals can be defined as compounds containing an unpaired electron, (Grisham 1992, Rice-Evans 1991).

The specific goals for this project were twofold: (a) to detect the free radicals generated by TCE ; (b) to determine a means of quantitating these radicals . TCE is widely used by the USAF as a degreasing agent because it is lipophilic and does not damage metal alloys. TCE decomposes by free radical pathways (von Sonntag and Schuchmann 1991). It is hoped that the study of the initial

free radical(s) generated by TCE may provide a better understanding of the role radicals or their metabolites play in hepatotoxicity.

This is the initial study of the radicals of TCE necessary to establish a simple, sensitive, and reliable assay to determine the level and extent of liver damage caused by exposure to TCE. Radicals can be generated by hydrolysis, heat, radiation, ultrasound as well as biological decomposition by enzymes (Rice-Evans et al. 1991). TCE radicals were generated in this project by γ -radiation. To meet the objectives of detecting TCE radicals, the techniques of electron paramagnetic resonance (EPR) and spin trapping were used.

EPR spectroscopy measures the effect of a magnetic field on an unpaired electron (free radicals and transition metals). The spinning electron acts as a small magnet. It also interacts with neighboring nuclei. When placed in an external magnetic field, information is obtained regarding the local environment surrounding the unpaired electron. The number of EPR lines which will be obtained can be predicted from equation 1:

$$\text{Equation 1} \qquad \text{EPR lines} \qquad = \qquad 2 I + 1$$

where I is the nuclear spin. TCE is a chlorinated hydrocarbon. Carbon has a nuclear spin of 0 and can form one line on the EPR spectra and hydrogen has a nuclear spin of one half and can form two lines on the EPR spectrum. EPR is the most sensitive and direct method for studying free radicals and it provides information on the molecular environment surrounding the unpaired electron (Mason 1982, Kalyanaraman & Sivarajah 1984 and Cavalieri & Rogen 1984). The EPR facility at the Armed Forces Radiobiology Research Institute, Bethesda, MD has two EPR spectrometers modified to measure free radicals in biological tissues.

Spin trapping is the reaction of a short lived free radical with a spin trap, yielding a longer lived nitroxide spin adduct which can be measured and identified by EPR. There are a number of spin traps available (Mason 1984). Spin traps are usually nitroso or nitron compounds. The nitrogen of the trap has a nuclear spin of one, yielding three lines on the EPR spectrum. The spin traps chosen for this study were the nitron traps N-tert-butyl- α -phenyl nitron (PBN) and 5,5-dimethyl-1-pyrroline-1-oxide (DMPO) and the nitroso spin trap 3,5-dibromo-4-nitrosobenzenesulphonate (DBNBS). These chemicals can trap carbon-, oxygen- and nitrogen-centered free radicals.

Thus EPR/spin trapping was used to detect the TCE radical produced after irradiation. The data obtained from the EPR spectrum was used to establish the trapping efficiency of the PBN trap which is suitable for use with biological tissue.

SECTION 2

MATERIALS & METHODS

Chemicals

All chemicals were Analar grade. N-tert-butyl- α -phenyl nitron (PBN), and 5,5-dimethyl-1-pyrroline-1-oxide (DMPO) were obtained from Aldrich Chemical Co. and 3,5-dibromo-4-nitrosobenzenesulfonate (DBNBS) was obtained from Sigma Chemical Co.

Radiation Procedure

Trichloroethylene in water (50%TCE: 50%water) were irradiated with spin traps. The concentration of the spin traps was 10mM. The solutions were irradiated in a ^{60}Co γ -ray source at a rate of 1Gy/min. receiving a total dose of 10 Gy.

To determine the trap efficiency TCE containing the spin trap PBN was γ -irradiated (10 Gy).

Double integration of the PBN-TCE adduct EPR is directly proportional to the number of radicals trapped. The number of radicals formed was estimated from the γ -ray energy deposition into the sample and the C-Cl bond energy. The efficiency of the trap is calculated from equation 2:

$$\text{Equation 2} \quad \text{Trapping efficiency} = \frac{\# \text{ radicals trapped}}{\# \text{ radicals formed}} \times 100$$

Electron paramagnetic resonance spectroscopy

First derivative spectra of irradiated TCE were measured at 20°C using a Varian E109 spectrometer and the spectrum were simulated by computer program using methods previously described (Carmichael et al 1993). The trapping efficiency was determined from the EPR spectra obtained from a Bruker ESP300 spectrometer.

SECTION 3

RESULTS

Detection of TCE Radicals

Trichlorethylene is a chlorinated hydrocarbon. Radicals of TCE are generated with radiation. We were able to detect TCE radicals after γ -irradiation. A total radiation dose of 10 Gy provided sufficient energy to the TCE molecule so that it released a bond and formed a carbon-centered radical, TCE•. This carbon-centered radical is very short lived and was reacted with two nitron and one nitroso spin trap to yield a longer lived radical adduct which was identified by EPR.

1. Nitron spin trap

The TCE radical adducts and the computer simulated spectra with the nitron traps , DMPO and PBN are shown in Figures 1 and 2 respectively. Both these traps indicate irradiation of TCE produces a carbon-centered radical, TCE•.

a. DMPO

The first derivative DMPO-TCE radical adduct is shown in Figure 1A and Figure 1B. The hyperfine coupling constants were measured directly from the spectrum. The hyperfine coupling constants of the DMPO-TCE spin adducts are $a_N = 1.40$, mT and $a_H^\beta = 1.95$ mT. The DMPO-TCE radical adducts show the nitrogen splitting with three lines. As expected, the spectrum in Figure 1A consists of a superimposition of two spin adduct EPR spectra since this spectrum was obtained after γ -irradiation of a TCE/water mixture. The more prominent spectrum consists of a 1:2:2:1 quartet ($a_N = a_H^\beta = 1.49$ mT) corresponds to the DMPO-OH adduct

originating from the radiation induced production of $\bullet\text{OH}$ radicals in water. The second EPR spectrum consists of a triplet of doublets originating from a carbon-centered radical on TCE. The data does not allow the distinction to be made whether this adduct originates from an initial TCE radical induced directly by the deposition of radiation energy, or whether it originates from the reaction of radiation induced $\bullet\text{OH}$ radicals with TCE. Since it was of interest to observe the initial TCE radicals formed, TCE was directly irradiated in the presence of DMPO and the EPR spectrum measured, Figure 2B. The computer simulation of this EPR spectrum $a_N = 1.40$, mT and $a_H^\beta = 1.95$ mT is shown in Figure 2C.

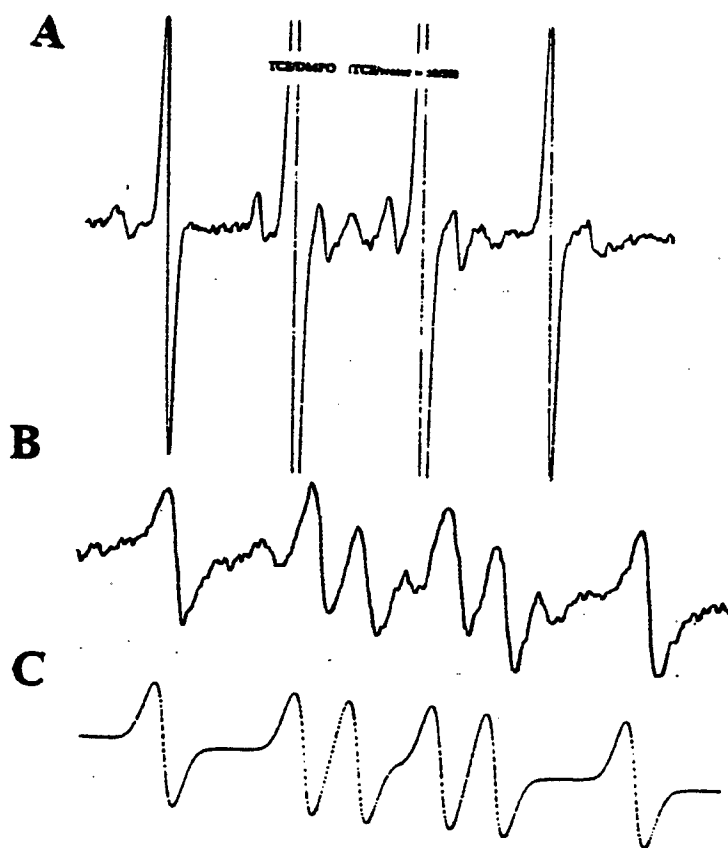
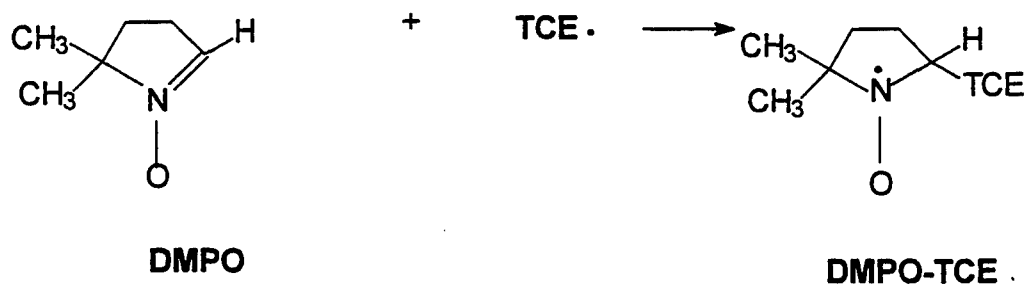


Figure 1. *The spectrum of DMPO-TCE radical.*

This data cannot provide any information on the structure of the TCE radical, Equation 3.

Equation 3



b. PBN Trap

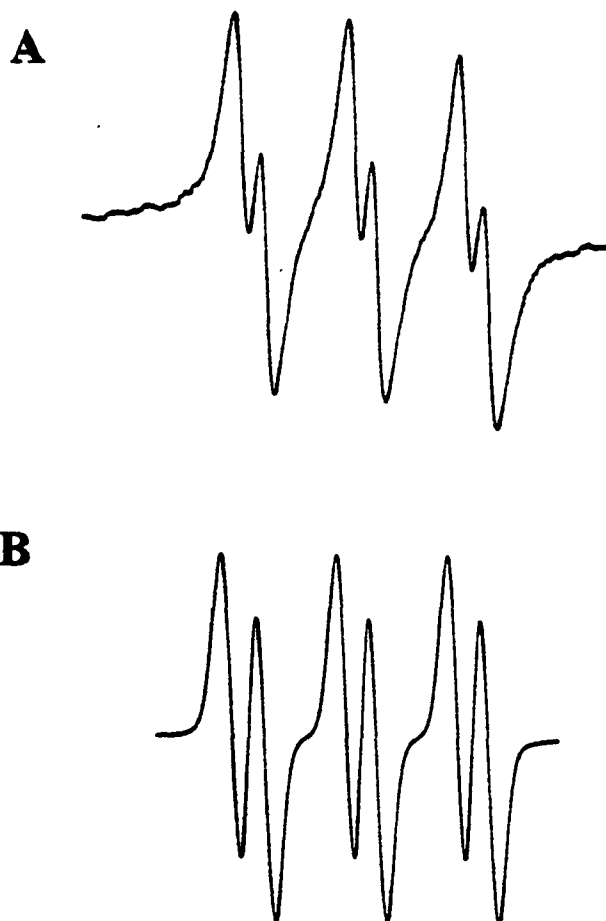
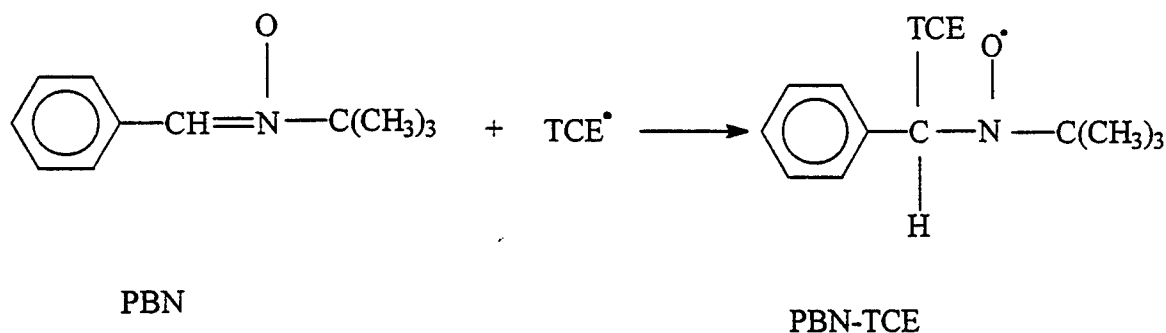


Figure 2. Spectrum of PBN-TCE radical.

The PBN-TCE first derivative spectrum is shown in Figure 2A. The equation of the PBN reaction with TCE is shown in Equation 4. The hyperfine coupling constants determined from the spectrum were $a_N = 14.25$ G and $a_H^\beta = 3.20$ G. The computer simulated spectrum is shown in Figure 2B. This spectrum is a typical triplet of doublets. The nitrogen has a nuclear spin of one giving a three line spectrum and the β -hydrogen has a nuclear spin of one half which splits the spectrum. The PBN trap like the DMPO trap can only confirm TCE forms a carbon-centered radical, Equation 4. However, because PBN is the least toxic spin trap to biological systems and will be the spin trap of choice for the biological studies, it is important to measure the adduct shown in Figure 2, for purposes of estimating trapping efficiency which is also required for biological studies.

Equation 4



2. Nitroso Spin Trap

The nitroso spin trap provides more information about the structure of the TCE radical. The DBNBS-TCE radical adduct is shown in Figure 3A. From this spectrum we determined the hyperfine coupling constants of the primary nitrogen, the β -hydrogen and the ring-hydrogen of the DBNBS. These hyperfine coupling constants were: $a_N = 14.0$ G and $a_H^\beta = 14.0$ G and $a_{H(2)}$

= 0.75 G respectively. The computer simulated spectrum shows four groups of lines because the β -hydrogen and the primary nitrogen have equal splitting. The two center lines in the quartet were of equal intensity (1:2:2:1). Each ring proton splits each line in this quartet into doublets. As the ring protons are equivalent and have equal splitting the final spectrum has a 1:2:1 triplet. The complete spectrum is a 1:2:2:1 quartet with each line of the quartet split into a 1:2:1 triplet, Figure 3B.

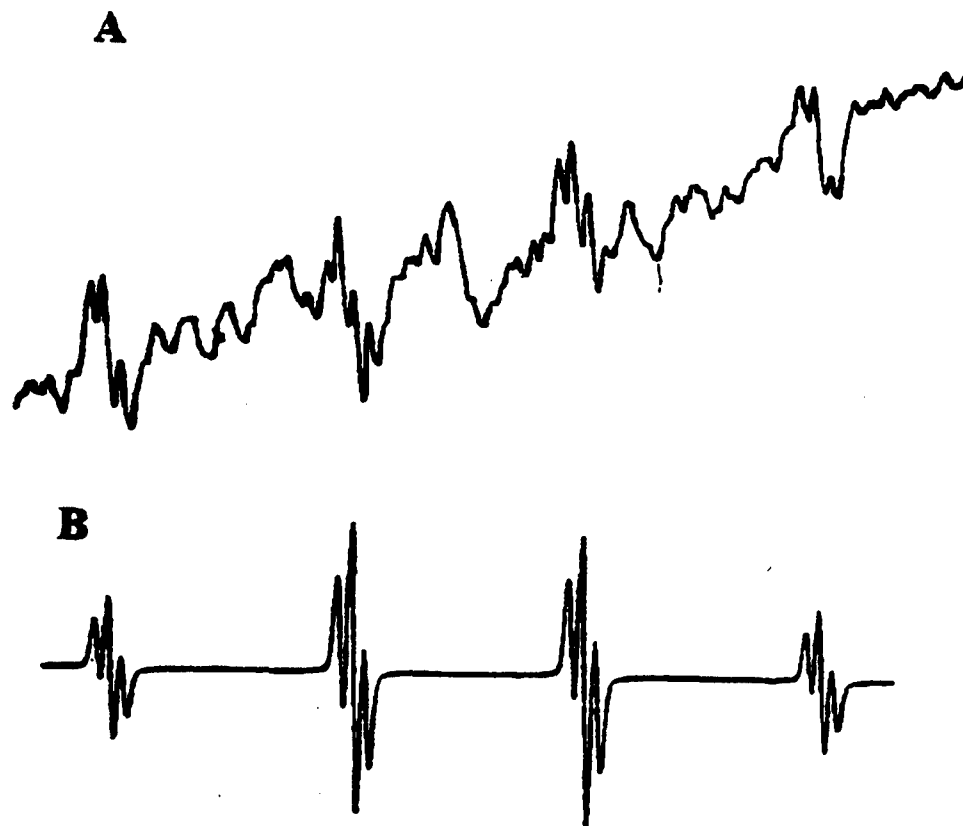


Figure 3 *Spectrum of DBNBS-TCE radical*

Figure 4 shows the possible radical structures of TCE.

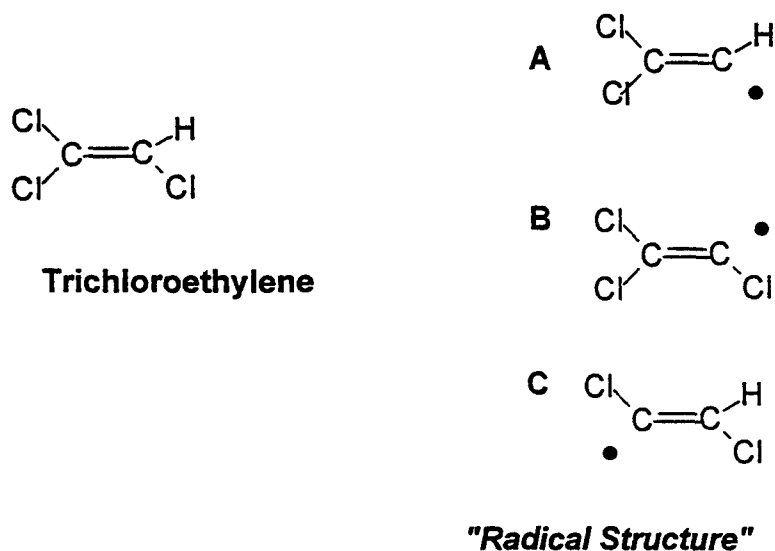


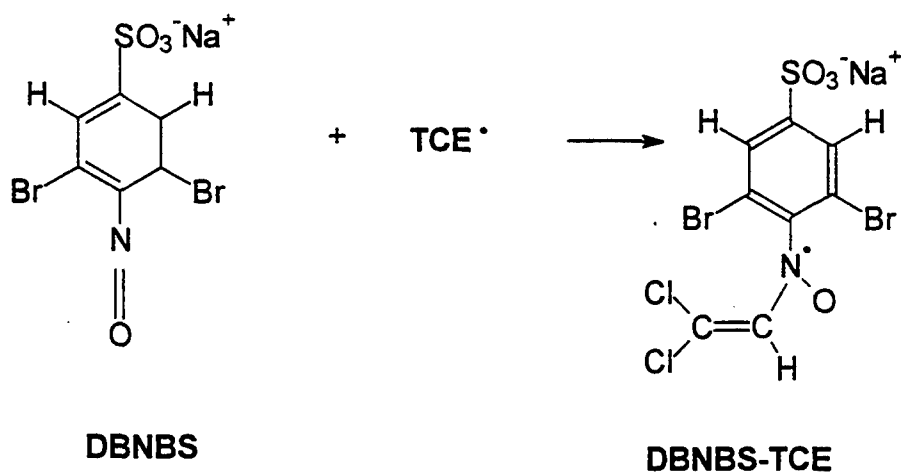
Figure 4 *Structure of trichloroethylene and possible radicals.*

From the three possible structures only one can produce the spectrum found with DBNBS.

Figure 4A is the only radical which will give a β -hydrogen with the nitroxide of DBNBS.

Therefore Equation 5 has been written showing the DBNBS-TCE radical adduct with the TCE structure shown in Figure 4A.

Equation 5



B. Measurement of Trap efficiency

The estimated trapping efficiency is given in Table 1. Using equation 2 the trapping efficiency for TCE radicals was estimated to be 20.6%. These calculations are based on the energy given to the TCE by the radiation and the energy required to break the carbon-hydrogen and carbon-chlorine bonds.

Spin Trap PBN	Value
C-Cl bond dissociation energy	3.49 eV
Energy from γ -rays	6.242×10^{17} eV/g
Energy input into the system	9.113×10^{16} eV
Maximum possible bonds broken	2.611×10^{16}
Maximum possible radicals formed	2.611×10^{16}
Number of radicals trapped by PBN	5.376×10^{15}
PBN trapping efficiency of TCE radicals	20.6%

Table 1. Estimated trapping efficiency for TCE radicals with PBN spin trap.

DISCUSSION

The environment has always naturally imposed limits on living cells (Williams, 1995). Most biological products contain carbon, so it is important to understand the chemistry of carbon compounds, to relate them to their biological effects in living cells (Grundon and Henbest 1971). A biological cell contains approximately 70% water and a wide range of organic substances. Thus it is important to study biological reactions in the aqueous environment. The elements nitrogen, sulfur and phosphorus as well as hydrogen and oxygen are present in many biological compounds. This is understandable because these elements have always dominated our environment. Plant and animal cells also contain inorganic ions which are believed to be essential for normal health: Na, Mg, Cl, K, Ca, Mn, Fe, Co, Cu, Zn, Mo, V, Cr, Se, Ni, B, I, F (Williams 1995). Normal function of a living cell therefore depends on a delicately poised complex of chemical reactions.

Free radical reactions dominate living cells because oxidative cross-linking of polymers by radical reactions is required to generate multicellular life. The best way to study radical reactions which occur in cells is to try to reproduce them in a test tube. Any compound which is irradiated will form free radicals (Rice-Evans 1991). Radiation is therefore a useful tool to generate radicals in controlled conditions. With controlled reactions, EPR can be used to elucidate the molecular structure of radicals.

TCE is a halogenated alkene. Irradiation of TCE by γ -radiation formed radicals which were detectable by EPR (Figures 1, 2, and 4). Structurally, TCE has a carbon-carbon double

SECTION 5

REFERENCES

- Carmichael, A.J., L. Steel-Goodwin, B. Gray., and C.M. Arroyo** 1993 Reactions of active oxygen and nitrogen species studied by EPR and spin trapping. *Free Rad Res Comms* 19 (1) S1-S16.
- Cavalieri, E.L. and E. G. Rogen** 1984 In: *Free Radicals in Biology Vol VI Chapter 5* (W.A. Pryor Ed.) Academic Press, NY
- Gonthier, B.P. and L.G. Barret** 1989 *In vitro* spin trapping of free radicals produced during trichloroethylene and diethyl ether metabolism *Tox Lett* 47: 225-234
- Grisham, M.B.** 1992 In *Reactive metabolites of oxygen and nitrogen in biology and medicine*. CRC Press, Boca Raton, Fl.
- Grundon, M.F. and H.B. Henbest** 1971 *Organic Chemistry An Introduction* MacDonald Technical & Scientific, London UK
- Kalyanaraman, B and K. Sivarajah** 1984 In *Free Radicals in Biology Vol VI Chapter 5* (WA Pryor Ed) Academic Press
- Knecht, K.T., and R.P. Mason** 1988 *In vivo* radical trapping and biliary secretion of radical adducts of carbon tetrachloride-derived free radical metabolites. *Drug Met. Dis. Biol. Fate. Chem* 16 (6) : 813-817
- Knecht, K.T., and R.P. Mason** 1992 Inhibition of radical adduct reduction and reoxidation of the corresponding hydroxylamines in *in vivo* spin trapping and carbon tetrachloride-derived radicals *Free Rad Biol & Med* 13: 151-160
- LaCagnin, L.B., H.D. Connor, R.P. Mason and R.G. Thurman.** 1988 The carbon dioxide anion radical adduct in the perfused rat liver: relationship to halocarbon-induced toxicity. *Mol. Pharmacol* 33 (3): 351-357
- Mason, R.P.** 1982 In : *Free Radicals in Biology Vol V* pp 161-196 (WA Pryor Ed); Academic Press, NY
- Mason, R.P.** 1984 Spin trapping free radical metabolites of toxic chemicals. In *Spin Labeling in Pharmacology Chapter 2* pp 87-129

The second assumption is that in the biological system the concentration of the spin trap is sufficiently high to effectively compete and trap the radical generated.

- This can be controlled if the spin trap is nontoxic.
- This can be achieved if the reaction of radical metabolites can be kinetically driven to react with the spin trap rather than other constituents in the cells.

In conclusion, radiation was used to detect the structure of the TCE radical and the trapping efficiency of the spin trap PBN, a possible trap for biological samples, was estimated to be 20.6%.

bond and three chlorines, Figure 3. In theory, TCE can produce three radicals. Experimentally, we demonstrated that only one radical is formed, Figure 3C. The structure of the TCE radical was detected using the spin trap DBNBS.

Because the TCE radical is very short-lived it was necessary to immobilize the radicals by lowering the temperature to 77°K. In living mammalian cells reactions normally occur at 37°C, so we recommend that samples be frozen in liquid nitrogen at harvest. To concentrate TCE radicals generated biologically, we also suggest that the samples be lyophilized. The lyophilized sample can be read by EPR as a solid sample. EPR does not physically or chemically alter a sample unlike other analytical techniques. The solid samples can be used to quantitate radicals and if necessary they can be rehydrated for identification of radical adducts.

Using radiation, the trapping efficiency of the nitron spin trap PBN was determined. Based on these experiments, the trapping efficiency of PBN for the TCE• is 20.6%. Until there is a method to measure TCE• directly, this is the only available estimate of trapping efficiency.

The nitron spin trap, PBN, has been used both *in vivo* and *in vitro* to study radicals, (Mason 1982, Knecht & Mason 1992, Rice-Evans et al 1992). We suggest PBN be used as the spin trap in biological experiments. Use of PBN is based on two assumptions.

The first assumption is that initial radicals generated in the test tube are the same as those generated in the biological system. Most labile bonds will be broken in either system or the best leaving groups are the ones that will leave the molecule in both systems.

- Injecting energy into the pure compound will break most labile bonds or allow these groups to break off and leave.
- Irradiation of the pure TCE will yield sufficient energy for labile bonds to break.

Rice -Evans, C.A., A. T. Diplock and MCR Symons 1991 Techniques in free radical research
In Laboratory Techniques in Biochemistry and Molecular Biology (RH Burdon & PH van
Knippenberg Ed) Elsevier, NY

Sentjuri, M and R.P. Mason 1992 Inhibition of radical adduct reduction and reoxidation of the
corresponding hydroxylamines in *in vivo* spin trapping of carbon tetrachloride derived radicals.
Free Rad Biol & Med 13: 151-160

von Sonntag, C and H-P Schuchmann 1991 The elucidation of peroxy radical reactions in
aqueous solution with the help of radiation-chemical methods. Angew Chem Int Ed Engl 30:
1229-1283

Williams, R.J.P. 1995 Bringing Chemistry to Life Chemistry International 17 (2) : 45-49.